

IN THE SPECIFICATION

Please replace paragraph [0011] with the following amended paragraph:

[0011] A considerable expenditure on instrumentation is required in order to realize these suggestions. Further, the recording of additional images prolongs the required recording time and therefore also increases aging of the sample by illumination with the fluorescence excitation light.

Please replace paragraph [0019] with the following amended paragraph:

[0019] Fig. 1 is a simplified optical schematic illustrating the structured illumination with transmitted illumination by way of example. The imaging beam path (image-forming beam path) is shown. A one-dimensional periodic structure (transmission grating) (3) which is located in a conjugated object plane of the optical arrangement shown in the drawing is illuminated by a light source (1) through collector optics (2). The grating is followed in light direction by a plane-parallel glass plate (4). The angle of the plane-parallel plate relative to the optical axis can be adjusted in a defined manner. The structure is imaged in the specimen plane (7) by the illumination-side optics (5, 6) (condenser). Imaging is effected by the light coming from the specimen through a pair of lenses (8, 9) (objective and tube lens) in the image plane (10) following the latter in which, e.g., a CCD matrix of a digital camera can be arranged. By tilting it in a defined manner, the plane-parallel glass plate (4) serves to displace the image of the grating structure (3) on the object in the specimen plane (7). For incident light fluorescence observation, the objective (8) serves at the same time as a condenser. As was described in WO 02/12945, the resulting brightness distribution in the object is registered in at least 3 positions of the glass plate (4) by means of the digital camera. In this connection, the brightness distribution $I_i(x, y)$ for a sine/cosine grating can be stated in a simplified manner by the following formula:

$$I_i(x, y) = I_0(x, y) \cdot (1 + m(x, y) \cdot \cos(\phi_0(x, y) + \phi_i(x, y))), \quad (1)$$

where $i=0 \dots N-1$ is the i th phase position of the projected grating, and N is the number of recordings, $m(x, y)$ is the modulation depth of the object (and therefore the image information sought at point x, y), and ϕ_i are the phase values. This equation contains the three unknown variables I_0 , m and ϕ_0 . Accordingly, these variables can be determined by means of at least three measurements with specifically varied ϕ_i ($i = 1, 2, 3$). The solution can be found from the measurements through a least square formulation. For this purpose, it is possible to represent Equation (1) in a more compact form and to rewrite the cosine function using the addition theorem:

$$I(x, y, \phi_i) = a_0(x, y) + a_1(x, y) \cdot f_1(\phi_i) + a_2(x, y) \cdot f_2(\phi_i), \quad (2)$$

where

$$\begin{aligned} f_1(\phi_i) &= \cos \phi_i \\ f_2(\phi_i) &= \sin \phi_i \\ a_0(x, y) &= I_0(x, y) \\ a_1(x, y) &= I_0(x, y) \cdot m(x, y) \cdot \cos \phi_0(x, y) \\ a_2(x, y) &= -I_0(x, y) \cdot m(x, y) \cdot \sin \phi_0(x, y) \end{aligned}$$

Please replace paragraph [0020] with the following amended paragraph:

[0020] The functions f_1 and f_2 therefore depend only on the phase displacements ϕ_i , which can be freely selected in principle. For a structure that is not sinusoidal but is periodic, the brightness distribution $I_i(x, y)$ can also be approximated by series expansion. The principle of calculation remains basically the same. Expressed in matrix form, the least square solution is:

$$\hat{M} \cdot \vec{a} = \vec{b}, \quad (3)$$

where

$$\hat{M} = \begin{pmatrix} N & \sum_N f_1(\phi_i) & \sum_N f_2(\phi_i) \\ \sum_N f_1(\phi_i) & \sum_N f_1^2(\phi_i) & \sum_N f_1(\phi_i)f_2(\phi_i) \\ \sum_N f_2(\phi_i) & \sum_N f_1(\phi_i)f_2(\phi_i) & \sum_N f_2^2(\phi_i) \end{pmatrix}, \quad (4)$$

where N is the number of measurements (in this case, phase steps) and

$$\vec{a} = \begin{pmatrix} a_0(x, y) \\ a_1(x, y) \\ a_2(x, y) \end{pmatrix} \quad (5)$$

and

$$\vec{b} = \begin{pmatrix} \sum_N I(x, y, \phi_i) \\ \sum_N [I(x, y, \phi_i) \cdot f_1(\phi_i)] \\ \sum_N [I(x, y, \phi_i) \cdot f_2(\phi_i)] \end{pmatrix}$$

structure (3) are designated by (12), the zero point is designated by (13) and corresponds to the DC component of the illumination, i.e., a uniform, unstructured illumination.

Please replace paragraph [0029] with the following amended paragraph:

[0029] For this purpose, a reference image (object with superimposed grating) is initially recorded and registered. A slight phase displacement of the grating to an initially unknown phase position of the grating is then carried out (this "displacement per control signal" is to be calibrated first) and a new image recording is made. The two images that are obtained are then compared by computation. Without limiting generality, this can be carried out by subtracting, summing, or in any other way that results in a merit function. The initial result is generally a striped image. The steps comprising displacement of the grating by a small amount, image recording, and comparison to the reference image are repeated until the merit function reaches the extreme value, that is, e.g., a difference image comprises only background noise or a sum image reaches maximum values. When the latter is accomplished, the calibration is finished and a control value obtained in this way can be kept in a storage medium so that it can be retrieved at a later time. Therefore, this value corresponds exactly to the displacement of the grating by a full period. Alternatively, it is also possible to assess a sum image for uniform brightness distribution (i.e., disappearance of stripe structure). In this case, this value corresponds to the displacement of the grating by half a period. This procedure is preferably carried out with a mirror as object. The described process can be carried out manually or automatically.

Please replace paragraph [0034] with the following amended paragraph:

[0034] The vector $\underline{0} < \mathbf{k}_i \leq \underline{1}$ comprising spatially varying factors describes the attenuation as a function of the shape of the irradiation intensity and a selectable quantity $1 > d \geq 0$ for the bleaching when multiplied component-wise by the observations o_i that have not yet undergone bleaching:

$$g_i = o_i k_i \quad (11)$$

Please replace paragraph [0035] with the following amended paragraph:

[0035] It follows from (10) that every observation is always multiplied by the factors of all preceding phase recordings; the latter are already bleached in part and overlap with the instantaneous phase. Alternatively, k_i can also be described exponentially, i.e., corresponding to the fluorescence decay curve of a particular fluorophore.

Please replace paragraph [0036] with the following amended paragraph:

[0036] Non-sinusoidal grating shapes cause stripe artifacts particularly at integral multiples of the grating frequency. With exact knowledge of frequency, phase position and curve shape of the grating in the individual recordings, it is possible to generate a sinusoidal shape. A correction vector I_{Corr} similar to (10), but in this case along the grating periodicity, is considered in (12).

$$I_{Corr} = \frac{f_{sin}}{f_{grating} + s} \quad (12)$$

Please replace paragraph [0041] with the following amended paragraph:

[0041] The merit function (Equation (16)) is mentioned here only by way of example. Other merit functions are also applicable. The aim is to vary θ_i , d and b in such a way that (16) is minimized. Many methods are available for this purpose which are by no means limited to gradient methods or line-search methods. Further, every transformation coefficient is weighted by a coefficient α_i . Accordingly, the algorithm can be adapted to different signal-to-noise ratios or preferred frequencies. An advantageous value in this respect for weighting the DC component is given by the following equation:

$$\alpha_0 \propto \frac{|F\{\bar{a}\}_1|^2 + |F\{\bar{a}\}_2|^2 + \dots + |F\{\bar{a}\}_n|^2}{|F\{\bar{a}\}_0|^2} \quad (17)$$

Please replace paragraph [0044] with the following amended paragraph:

[0044] Another approach for taking into account bleaching effects in the object in fluorescence illumination is based on determination of a local correction function. For this purpose, a correction function $\kappa_i(x)$ is assumed:

$$\kappa_i(x) = \frac{\int_{x-\tau/2}^{x+\tau/2} g_1(\xi) d\xi}{\int_{x-\tau/2}^{x+\tau/2} g_i(\xi) d\xi}, \quad (18)$$

whose local value is formed by integration over the grating period $\tau = 2\pi/\omega$. The formulation of this function can also take into account the dependency on y , although this is omitted in the present case for the sake of simplicity. Using a spatially variable bleaching function $\theta_i(x)$ and by suitable conversions with the approximation (see also formulas (1) and (9)

$g_i(x) = \theta_i(x)I_0(x)[1 + m \cos(\omega x + \varphi_i)]$, it can be shown by the following equation

$$\kappa_i(x) = \frac{\int_{x-\tau/2}^{x+\tau/2} I_0(\xi) d\xi + m \int_{x-\tau/2}^{x+\tau/2} I_0(\xi) \cos(\omega \xi + \varphi_1) d\xi}{\int_{x-\tau/2}^{x+\tau/2} \theta_i(\xi) I_0(\xi) d\xi + m \int_{x-\tau/2}^{x+\tau/2} \theta_i(\xi) I_0(\xi) \cos(\omega \xi + \varphi_1) d\xi}$$

that this correction function is proportional to $1/\theta_i(x)$ with uniform bleaching function.

Please replace paragraph [0046] with the following amended paragraph:

[0046] For this purpose, the threshold ε is advantageously set as a percentage of the variation of the local variability of the correction function within the image, e.g., 5%. Instead of $1/\theta_i$, interpolated values of $\kappa_i(x)$ which were calculated below the threshold in the two-dimensional space can be used outside the threshold.